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14. ABSTRACT

The main objective of this project is to evaluate the effects of soy phytoestrogens on reproductive hormones and prostate tissue markers of cell proliferation and androgen action in men at high risk of prostate cancer. The hypothesis is that alteration of endogenous hormones is a mechanism by which soy phytoestrogens prevent prostate cancer. A randomized parallel arm study is being performed, in which 63 men at high risk of prostate cancer are randomized to receive one of three dietary supplements for six months: 1) soy powder containing phytoestrogens; 2) phytoestrogen-free soy powder; or 3) phytoestrogen-free milk powder. Urine and blood is collected at 0, 3 and 6 mo, for evaluation of serum hormones (testosterone, dihydrotestosterone, androstenedione, dehydroepiandrosterone, estradiol, estrone, 3α,17β-androstanediol glucuronide, sex hormone binding globulin) and prostate specific antigen, as well as urinary estrogen and phytoestrogen metabolites. At 0 and 12 mo, prostate biopsies are performed to evaluate prostate tissue expression of apoptosis (TUNEL assay, Bax, Bcl-2), proliferation (Ki67, PCNA), and androgen receptor density. A pilot study is being performed to evaluate effects on protein expression in biopsy tissue and phytoestrogen levels in expressed prostatic secretion and post-massage urine. The main study is complete: one manuscript has been accepted for publication, two other are under revew, and one is preparation. The pilot study is continuing with funding from the University of Minnesota.

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Prostate cancer, phytoestrogen, soy

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INTRODUCTION

The low risk of prostate cancer in Asia is thought to be due to dietary factors, including soy consumption. Studies showing an inverse association between prostate cancer risk and urinary excretion of soy phytoestrogens suggest that phytoestrogens contribute to the cancer-preventive effects of soy. One mechanism by which soy phytoestrogens are thought to be cancer-preventive is *via* reduction of endogenous sex hormones known to stimulate prostate cell growth. Despite the interest in soy phytoestrogens for prevention of prostate cancer, there have been no studies in men to evaluate the effects of soy phytoestrogen consumption on sex steroids and prostate tissue biomarkers, and no studies evaluating effects of phytoestrogen metabolism on sex steroids in men.

The main objective of this project is to evaluate the effects of soy phytoestrogen consumption on reproductive hormones and prostate tissue markers of cell proliferation and androgen action in men at high risk of prostate cancer. The underlying hypothesis is that alteration of endogenous hormones is a mechanism by which soy phytoestrogens prevent prostate cancer.

The specific aims of this study (SoyCaP) are to compare the effects of consumption of phytoestrogen-containing soy protein, phytoestrogen-free soy protein, and milk protein, on risk factors for prostate cancer (endogenous hormones, prostate specific antigen, prostate tissue markers of cell proliferation and hormone action), in men at high risk for prostate cancer. Comparing the three groups will enable us to distinguish the specific effects of soy phytoestrogens from effects caused by other soy components. A randomized parallel arm study will be performed, in which 63 men at high risk of prostate cancer will be randomized to receive one of three dietary supplements for six months: 1) soy powder containing 1 mg phytoestrogens/kg body weight; 2) phytoestrogen-free soy powder; and 3) phytoestrogen-free milk powder. Urine and blood will be collected at 0, 3 and 6 months, for evaluation of serum hormones (testosterone, dihydrotestosterone, androstenedione, dehydroepiandrosterone, estradiol, estrone, 3α, 17β-androstanediol glucuronide, sex hormone binding globulin) and prostate specific antigen, as well as urinary estrogen and phytoestrogen metabolites. Before and after the intervention, prostate biopsies will be performed to evaluate prostate tissue expression of apoptosis (terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) assay, Bax, Bcl-2), proliferation (Ki67, proliferating cell nuclear antigen (PCNA)), and androgen receptor density.

Data from *in vitro*, animal and epidemiological studies suggest that androgens and estrogens play a role in prostate carcinogenesis. Soy phytoestrogens have been shown to alter sex steroids in women in a potentially beneficial direction, yet such studies in men have not been reported. Studies of the hormonal effects of soy phytoestrogens in men will contribute to our knowledge of the cancer-preventive mechanisms of soy phytoestrogens, and may lead to dietary recommendations for prevention of prostate cancer.

BODY

According to the original statement of work, the following tasks were to be performed during the three years of this project:

Task 1: Hire and train staff, coordinate with Veteran's Administration and Fairview-University Hospital staff, establish all study protocols

Task 2: Perform feeding study on 63 men

- Recruit 63 men at high risk of prostate cancer and randomize into three intervention groups: phytoestrogen-containing soy protein (Soy +), phytoestrogen-free soy protein (Soy-), or milk protein
- Perform feeding study; process and store serum, urine and biopsy slides
- Analyze samples: serum hormones and sex hormone binding globulin (SHBG) by radioimmunoassay (RIA); serum free and total prostate specific antigen (PSA) by enzyme-linked immunosorbent assay (ELISA); urine estrogen metabolites and phytoestrogens by GC-MS; biopsy slides by immunohistochemistry

Although the grant officially began on April 15, 2002, final approval from the DOD Institutional Review Board (IRB) was not received until January 2003. As a result, we were not able to begin recruiting subjects until February 2003 and began the study about one year late.

High PSA with negative prostate biopsy ("false positives") was a major enrollment criterion. Since being awarded the grant, the medical community decided that there are too many false positives, and is therefore not using PSA screening as widely as before. As a result, our pool of potential subjects was drastically lowered and recruitment has been difficult. Power calculations were performed under the guidance of Dr. William Thomas, to determine the minimum number of subjects required to evaluate our main endpoints. Dr. Thomas, a biostatistician, determined that 21 subjects in each arm would allow us to detect a 16% to 33% change in serum total testosterone (see Final Report 5/05). Thus we lowered the recruitment goal from 90 to 63, or 21 subjects per group.

Recruitment Summary

May 2005 - April 2006:

From May 2005 - April 2006, 27 subjects enrolled in the study (2-3 subjects/month, Table 1). Out of these 27 individuals, 3 individuals (11.2%) withdrew as a result of inconvenience or gastrointestinal discomfort (belching & bloated feeling).

Table 1a. Enrollment Summary 5/05-4/06

	Completed 6 months	Currently completing study	Withdrew after starting	Consented but did not start	Total enrollment
As of 4/05 (Previous DOD report)	32	6	7	17	62
5/05 – 4/06	18	7	3	5	27*
TOTAL	50	7	10	22	89

* The sum of the numbers across the rows in this enrollment period (33) includes the 6 people who were in the "currently completing study" group of the previous period and hence does not equal the total enrollment for that period which was 27 participants. Of the 27 subjects enrolled, another 5 individuals (18.5%) did not start the study as a result of inconvenience or placement on a physician monitored weight-loss plan. The overall drop-out rate during this period was lower than previous years (29.6% versus 38.7%, Table 1). We believe this increased success with enrollment is due in part to the monetary compensation we are now providing the subjects.

May 2006- April 2007

From May 2006 to April 2007, 2 subjects were enrolled in the study. One participant enrolled during the previous period dropped out during this period as he needed to be on a physician monitored weight loss plan.

Table 1b. Enrollment Summary 5/06-4/07

	Completed 6 months	**Currently completing study**	**Withdrew after Starting**	**Consented but did not start**	**Total Enrollment for the period**
As of April 2006 (Previous DOD report summary)	50	7	10	22	89
May 2006 - April 2007	6	2	0	1	**2***
TOTAL	**56**	**2**	**10**	**23**	**91**

* The sum of the numbers across the rows in this enrollment period i.e. 9 includes the 7 people who were in the "currently completing study" group in the previous period (one subject dropped out) and hence does not equal the total enrollment for that period which was 2 participants.

Entire Study: February 2003 - April 2007
From the start of the study to 04/30/2007, 91 subjects have enrolled in the study. Of these:
7 subjects dropped out before 3 months
23 subjects did not start the study after consenting
3 subjects completed 3 months of the study [2 Soy (+); 1 Soy(-)]
56 people have complete 6 months of the study [18 Soy (+); 19 Soy (-); 19 Milk]
2 people are current enrolled [2 Soy (+)]

Expansion of the Workscope: Additional Sample Collections
During the period of 4/15/05 to 4/14/06 we received IRB approval and have started collecting additional biological samples from the SoyCaP participants for a pilot study. The PI received a small grant from the University of Minnesota to fund the additional analyses. A summary of the rationale, collection methods and number of samples obtained as of 4/14/06 is provided below.

A. Additional Prostate Tissue Samples (For Proteomic Analyses):

Rationale
In-vitro data suggests that isoflavonoids alter gene transcription to affect prostatic cell proliferation and apoptosis, thus preventing transformed cells from progressing to clinically detectable tumors. However there are no data on whether similar effects are observed in men consuming soy. Additionally most data are based on gene transcription which may not reflect cell protein concentrations. Since cell proteins are more immediate effectors of cellular function, they are likely to provide a more accurate picture of the effects of isoflavonoid on prostate cell proliferation.

Sample Collection and Analyses
Tissue samples are obtained when participants undergo their study-scheduled biopsy i.e. after being on the intervention for 6 months. For the proteomics study, 4 additional cores are obtained, 2 from each side of the prostate. Comparative analysis of the protein profile in samples obtained from the 3 intervention groups will be performed using iTRAQ. Further proteomic analyses will be used to confirm the proteins and to identify candidate proteins that are likely to be altered by isoflavonoid and/or soy exposure.

Sample Numbers
As of 4/14/06, we have cores obtained from 13 participants. Of these 5 are from the Soy (+) group, 4 are from the Soy (-) group, and 4 are from the milk group.

B. Expressed Prostatic Secretion (EPS) and Post-Massage Urine (PMU)

Rationale:
Levels of phytoestrogens and hormones in the EPS are a better indicator of prostate tissue exposure than that in the plasma and/or urine. Yet few studies have actually measured the concentrations of phytoestrogens and hormones in the EPS. Also, no

data exist on the correlation of the levels of these metabolites in the EPS and that in urine or plasma. Since prostate cell behavior is determined by the concentration of these compounds at the prostate tissue level, determining their concentration in the EPS is important. By estimating the correlation between the concentration of phytoestrogens and hormones in the EPS and 24-hour urine and serum, we will be able to assess which biological fluid (24-hour urine or blood) provide more accurate data on the concentration of these metabolites at the prostatic level. Since post massage urine (PMU) contains prostatic secretion, it is possible that it correlates more closely with levels in the EPS than that in blood or 24-hour urine. PMU unlike EPS can be obtained from most subjects and would be more feasible to collect than EPS in future clinical studies.

Sample Collection and Analyses:
EPS and PMU is being collected at each clinic visit by Mr. Ross Haller, Dr. Joel Slaton's urology assistant. Mr. Haller is very experienced in collecting EPS samples and has been responsible for their collection in several other studies.

- EPS and post-massage urine obtained at baseline and from subjects on the Soy (+) arm of the study will be analyzed for isoflavonoids.
- EPS and post-massage urine obtained from subjects on the Soy (-) and milk arms of the study.

Sample numbers (as of 4/14/07):
Proteomics:
Total Numbers: 21 [7 Soy (+); 6 Soy (-); 8 Milk]
The method for proteomic analyses of prostate tissue has been developed in collaboration with the Mass Spectrometry Consortium for the Life Sciences and Proteome Analysis Core Facilities. Sample analyses will begin summer 2007.

EPS:
Baseline: 11 [4 Soy (+); 3 Soy (-); 4 Milk]
3-month visit: 5 [1 Soy (+); 4 Soy (-);]
6-month visit: 4 [1 Soy (-); 3 Milk]

Post-Massage Urine:
Baseline: 14 [6 Soy (+); 2 Soy (-); 6 Milk]
3-month visit: 13 [5 Soy (+); 3 Soy (-); 5 Milk]
6-month visit: 8 [2 Soy (+); 3 Soy (-); 3 Milk]
Quantification of isoflavone concentration in post-massage urine samples is ongoing.

Results

Dietary Data and Anthropometrics*:* No differences were observed in baseline anthropometrics, cancer status and dietary intake (Table 2), except that the SPI (-) group had higher baseline intake of protein, zinc and calcium, and the MPI group had a higher baseline body weight (Table 3).

Dietary intake of protein calcium and vitamin-D intake increased in all groups and were significantly higher than baseline values. Additionally, fat intake was reduced in the SPI (-) group at 3 months (Table 3). However, these dietary and anthropometric differences between groups were unrelated to changes in serum hormone concentrations and prostatic steroid-receptor expression profiles.

TABLE 2: Baseline characteristics of subjects [1]

	SPI (+)	**SPI (-)**	**MPI**
	n = 20	n = 20	n = 18
Age (y) Age (y)	68 ± 8	68 ± 5	68 ± 7
Body wt (kg)	91 ± 16 *ab*	88 ± 12 *a*	98 ± 15 *b*
Height (cm)	175 ± 7	173 ± 8	176 ± 8
BMI (kg/m^2)	30 ± 5	29± 4	32 ± 6
Prostate Cancer Markers [2]			
PIN (*n* (%))	18 (90)	18 (90)	14 (78)
ASAP (*n* (%))	3 (15)	7 (35)	4 (22)
CaP (*n* (%))	2 (10)	1 (5)	2 (12)

[1] All values are means ± SD except prostate cancer markers which are n (%).
[2] Prostate cancer markers PIN, ASAP, and CaP are not mutually exclusive.
[*ab*] Means in a row without a common letter differ ($p < 0.05$).

TABLE 3: Anthropometrics and dietary intake [1]

	SPI+ *n* = 20 [2]	**SPI-** *n* = 20	**MPI** *n* = 18
Weight (kg)			
Baseline	91 ± 16 *ab*	88 ± 12 *a*	98 ± 15 *b*
3 Mo	91 ± 16 *ab*	87 ± 12 *a*	98 ± 15 *b*
6 Mo	90 ± 16 *ab*	87 ± 13 *a*	99 ± 15 *b*
Height (cm)			
Baseline	175 ± 16	173 ± 8	176 ± 8
BMI (kg/m^2)			
Baseline	30 ± 5	29 ± 4	32 ± 6
3 Mo	30 ± 5	29 ± 4	32 ± 6
6 Mo	30 ± 5	29 ± 4	32 ± 6
Energy Intake (kcal/d) [3]			
Baseline	2140 ± 620	2260 ± 660	2070 ± 520
3 Mo	2220 ± 720	2030 ± 390	2180 ± 510
6 Mo	2240 ± 410	2120 ± 670	2330 ± 410
Protein (g /d)			
Baseline	83 ± 21 *a*	100 ± 24 *b*	81 ± 25 *a*
3 Mo	* 118 ± 24	* 117 ± 16	* 121 ± 30
6 Mo	* 118 ± 21	* 124 ± 29	* 120 ± 18
Carbohydrate (g/d)			
Baseline	256 ± 106	262 ± 118	236 ± 59

3 Mo	246 ± 97	230 ± 82	232 ± 75
6 Mo	251 ± 61	232 ± 89	256 ± 68
Total Fat (g/d)			
Baseline	86 ± 33	93 ± 32	88 ± 24
3 Mo	80 ± 39	* 74 ± 18	73 ± 30
6 Mo	83 ± 34	80 ± 34	89 ± 26
Saturated Fat (g/d)			
Baseline	27 ± 11	34 ± 14	28 ± 11
3 Mo	27 ± 13	* 26 ± 7	24 ± 12
6 Mo	26 ± 10	29 ± 14	30 ± 10
Cholesterol (mg/d)			
Baseline	324 ± 202	382 ± 153	301 ± 163
3 Mo	307 ± 131	296 ± 115	312 ± 233
6 Mo	328 ± 147	348 ± 175	329 ± 234
Fiber (g/d)			
Baseline	17 ± 9	18 ± 7	16 ± 5
3 Mo	16 ± 8	17 ± 8	15 ± 7
6 Mo	15 ± 9	16 ± 9	15 ± 5

Vitamin D (μg/d)			
Baseline	4 ± 3	4 ± 5	4 ± 3
3 Mo	* 9 ± 4	* 8 ± 3	* 8 ± 2
6 Mo	* 8 ± 2	* 8 ± 3	* 9 ± 2
Vitamin E (mg/d)			
Baseline	8 ± 7	8 ± 5	6 ± 4
3 Mo	6 ± 4	7 ± 10	6 ± 3
6 Mo	7 ± 7	6 ± 3	6 ± 3
Calcium (mg/d)			
Baseline	890 ± 400 *ab*	1230 ± 970 *b*	760 ± 360 *a*
3 Mo	* 2260 ± 440	* 2120 ± 350	* 2200 ± 380
6 Mo	* 2180 ± 290	* 2340 ± 840	* 2190 ± 340
Selenium (mg/d)			
Baseline	0.08 ± 0.05	0.09 ± 0.03	0.08 ± 0.05
3 Mo	0.08 ± 0.03	* 0.06 ± 0.03	0.10 ± 0.11
6 Mo	0.07 ± 0.03	* 0.07 ± 0.02	0.44 ± 1.6
Zinc (mg/d)			
Baseline	10 ± 6 *a*	14 ± 5 *b*	10 ± 5 *a*
3 Mo	11 ± 4	10 ± 8	10 ± 3
6 Mo	9 ± 3	10 ± 5	9 ± 3

[1] All values are means ± SD. [2] Sample sizes listed at column headings are for all time points except the following: 3 mo, MPI (n = 17), and 6 mo, SPI+ (n = 18) and SPI- (n = 18). [3] 1 kcal = 4.184 kJ [ab] Means in a row without a common letter differ ($P < 0.05$).
*Significant within-group change from baseline ($P < 0.05$).

Serum hormones, SHBG and receptor expression*:* Baseline serum hormone and SHBG concentrations and prostatic steroid hormone receptor expression levels did not differ between the groups (Tables 4 & 5). Six-month prostatic androgen receptor expression was lower in the SPI (+) group as compared to the MPI group (*P*=0.04) and tended to be lower in the SPI (-) group as compared to the milk group (*P*= 0.09). No differences were observed in estrogen receptor-beta expression (Table 3) Serum concentrations of estradiol, estrone, androstenedione and DHT increased during the intervention in the SPI (-) group, and at 3-months serum estrone and androstenedione concentrations were significantly higher in the SPI (-) group. These differences persisted at 6-months Also higher concentrations of estradiol, and DHEAS were observed at 6-months in the SPI (-) group (Table 4). Serum SHBG concentrations decreased from baseline in all 3 groups and no group differences were observed (Table 5).

TABLE 4: Steroid receptor expression (HSCORE) [1]

	SPI+	**SPI-**	**MPI**
Androgen Receptor (AR)			
Baseline	1.37 ± 0.06	1.28 ± 0.06	1.23 ± 0.06
6 Mo	1.26 ± 0.05 *a*	1.30 ± 0.05 *ab*	* 1.42 ± 0.05 *b*
Estrogen Receptor β (ERβ)			
Baseline	1.22 ± 0.06	1.32 ± 0.06	1.23 ± 0.06
6 Mo	1.16 ± 0.06	1.18 ± 0.06	1.26 ± 0.05

[1] Baseline data are unadjusted means ± SEM. All other data are least-squares means adjusted for baseline measurement ± SEM. The number of patients evaluated for AR expression was 14 for SPI+, 16 for SPI-, and 14 for MPI. The number of patients evaluated for ERβ expression was 14 for SPI+, 14 for SPI-, and 15 for MPI.
[ab] Means in a row without a common letter differ (*P* < 0.05).
*Significant within-group change from baseline (*P* < 0.05).

TABLE 5: Serum hormones and SHBG [1]

	SPI+ *n* = 20 [2]	**SPI-** *n* = 20	**MPI** *n* = 18
Estradiol (pmol/L)			
Baseline	67 ± 4	66 ± 4	69 ± 3
3 Mo	75 ± 5	* 76 ± 5	* 62 ± 6
6 Mo	69 ± 3 *a*	* 79 ± 3 *b*	66 ± 3 *a*
Estrone (pmol/L)			
Baseline	157 ± 15	141 ± 10	158 ± 8
3 Mo	150 ± 8 *ab*	* 170 ± 8 *b*	146 ± 8 *a*
6 Mo	152 ± 10	* 171 ± 10	150 ± 10
Androstenedione (nmol/L)			
Baseline	2.9 ± 0.3	2.9 ± 0.3	2.5 ± 0.2
3 Mo	3.0 ± 0.2 *a*	3.0 ± 0.2 *ab*	2.8 ± 0.2 *b*
6 Mo	2.6 ± 0.2 *a*	* 3.4 ± 0.2 *b*	2.9 ± 0.2 *ab*
Androstanediol Glucuronide (nmol/L)			
Baseline	19 ± 3	18 ± 5	16 ± 2
3 Mo	17 ± 2 *a*	24 ± 2 *b*	17 ± 2 *a*
6 Mo	16 ± 2	20 ± 2	18 ± 2
DHEAS (nmol/L) [†]			
Baseline	2202 ± 390	2052 ± 300	1977 ± 370
3 Mo	2040 ± 103 *a*	2715 ± 103 *b*	2126 ± 103 *a*
6 Mo	1937± 154 *a*	2372 ± 146 *b*	1946 ± 150 *a*

DHT (pmol/L)			
Baseline	1547 ± 190	1354 ± 170	1072 ± 110
3 Mo	1242 ± 81	* 1076 ± 79	1119 ± 100
6 Mo	1215 ± 94	1174 ± 89	1229 ± 105
Testosterone (nmol/L)			
Baseline	12 ± 1	13 ± 1	12 ± 1
3 Mo	13 ± 0.5	13 ± 0.6	11 ± 0.6
6 Mo	13 ± 0.6	13 ± 0.5	12 ± 0.6
Free Testosterone (pmol/L)			
Baseline	33 ± 3	34 ± 2	29 ± 2
3 Mo	33 ± 1	33 ± 1	32 ± 1
6 Mo	32 ± 1	32 ± 1	31 ± 1
SHBG (nmol/L) [‡]			
Baseline	63 ± 7	64 ± 8	69 ± 9
3 Mo	* 56 ± 3	* 56 ± 2	* 56 ± 3
6 Mo	* 54 ± 3	* 61 ± 3	* 58 ± 3

[1] Baseline data are unadjusted means ± SEM. All other data are least-squares means adjusted for baseline measurement ± SEM, except androstenedione which is additionally adjusted for interaction between treatment and baseline.
[2] Sample sizes listed at column headings are for all time points except: 3 mo MPI (*n* = 17), and 6 mo SPI+ (*n* = 18) and SPI- (*n* = 19).
[†] Sample sizes differed from other hormones due to excluded data. At 3 mo, SPI+ (*n* = 19) and SPI- (*n* = 19). At 6 mo, SPI+ (*n* = 17) and SPI- (*n* = 19).
[‡] Sample sizes differed from other hormones due to excluded data. At 3 mo, SPI+ (*n* = 19), and at 6 mo, SPI+ (*n* = 18).
[ab] Means in a row without a common letter differ ($P < 0.05$).
*Significant within-group change from baseline ($P < 0.05$).

Urinary Estrogen Metabolites: At baseline, urinary estrogen metabolites did not differ between the 3-groups with the exception of 2-methoxyestradiol which was significantly higher in the SPI (+) group as compared to the MPI group. At 3-months urinary estradiol and was significantly higher while 16α-hydroxyestrone tended to be higher in SPI (+) and SPI (-) groups as compared to the MPI group. These differences in urinary estradiol concentrations persisted at 6-months. Higher urinary 2-hydroxyestradiol levels were also observed in the soy groups as compared to the MPI group. The 6-month 2:16-hydroxyestrone ratio tended to be higher in the SPI (+) group as compared to the MPI group (Table 6).

Table 6: Urinary Estrogen Metabolites (nmol/day)

	SPI+	SPI-	MPI	P-Value
Sample size	*n = 19§*	*n = 19§*	*n = 17§*	
Estradiol (nmol/d)				
Baseline	50.5 (34, 74)	41.5 (27, 63)	48.7 (29, 81)	0.77
3 Mos	* 93.7 (65, 135) *a*	* 76.1 (53, 111) *a*	44.1 (30, 66) *b*	0.02
6 Mos	* 91.3 (63, 132) *a*	* 89.9 (63, 129) *a*	49.5 (34, 72) *b*	0.04
Estrone (nmol/d)				
Baseline	20.1 (15, 28)	18.1 (13, 26)	24.9 (20, 31)	0.92
3 Mos	25.6 (20, 34)	21.4 (16, 28)	21.6 (16, 29)	0.59
6 Mos	* 37.0 (28, 49)	* 26.8 (20, 35)	22.7 (17, 30)	0.06
2-methoxyestradiol (nmol/d)				
Baseline	57.4 (39, 84) a	42.1 (31, 56) ab	31.1 (21, 47) b	0.05
3 Mos	38.5 (26, 58)	42.0 (28, 62)	36.7 (24, 57)	0.90
6 Mos	26.2 (18, 38)	36.1 (25, 52)	34.3 (23, 50)	0.44
2-methoxyestrone (nmol/d)				
Baseline	9.05 (7, 12)	9.50 (6, 15)	8.10 (6, 12)	0.84

3 Mos	7.62 (5, 12)	9.53 (6, 15)	8.89 (6, 14)	0.76
6 Mos	9.82 (7, 15)	9.16 (6, 14)	7.94 (5, 12)	0.75
16α-hydroxyestrone (nmol/d)				
Baseline	5.98 (4, 9)	5.69 (4, 9)	6.90 (5, 10)	0.76
3 Mos	7.35 (5, 11) *a*	7.86 (5, 11) *a*	4.46 (3, 7) *b*	0.09
6 Mos	5.96 (4, 9)	7.30 (5, 11)	6.62 (5, 10)	0.72
2-hydroxyestradiol (nmol/d)				
Baseline	7.31 (5, 12)	4.79 (3, 8)	5.93 (3, 11)	0.47
3 Mos	7.18 (5, 11)	6.88 (4, 11)	5.61 (4, 9)	0.71
6 Mos	5.61 (4, 8) *a*	8.25 (6, 12) *a*	*3.00 (2, 4) *b*	0.001
2-hydroxyestrone (nmol/d)				
Baseline	19.8 (13, 30)	20.56 (13, 33)	25.8 (19, 36)	0.62
3 Mos	20.6 (14, 30)	23.4 (16, 34)	25.7 (17, 38)	0.71
6 Mos	28.9 (21, 41)	25.0 (18, 35)	21.3 (15, 30)	0.46
Estriol (nmol/d)				
Baseline	54.5 (42, 71)	28.4 (14, 57)	47.3 (29, 78)	0.14
3 Mos	28.3 (19, 42) *a*	56.3 (38, 84) *b*	40.8 (27, 63) *ab*	0.07
6 Mos	31.1 (19, 49)	45.2 (28, 72)	41.8 (26, 67)	0.50
2:16 (mean ± sd)				
Baseline	5.4 ± 1	6.0 ± 1	6.0 ± 1	0.92
3 Mos	5.9 ± 1	5.8 ± 1	8.2 ± 1	0.36
6 Mos	9.4 ± 2 *a*	6.1 ± 2 *ab*	4.0 ± 2 *b*	0.08

Baseline data are unadjusted geometric means ± 95% confidence intervals except 2:16 which are means ± standard errors. All other data are least-squares geometric means adjusted for baseline measurement ± 95% confidence intervals, except 16α-hydroxyestrone which is additionally adjusted for baseline weight. 2:16 data are least-squares means ± standard errors and were analyzed on the original scale. Pairwise baseline-adjusted comparisons between groups are within rows: means that do not share letters are significantly different ($p < 0.05$).
§ Sample sizes listed at column headings are for all time points except: 3-mo MPI: n=16, 6-mo SPI (+): n =17 and 6-mo SPI (-): n =18
*Significant within-group change from baseline ($p < 0.05$).
Abbreviations: SPI+ = soy protein isolate with isoflavones (40 g soy protein, 107 mg isoflavones); SPI- = soy with protein isolate very low isoflavones (40 g soy protein, < 6 mg/d); MPI = milk protein isolate (40 g milk protein); 2:16 = 2-hydroxyestrone: 16α-hydroxyestrone

Prostate Cancer Biomarkers, PSA and prostate volume: No between-group differences in baseline aggregate antigen expression HSCORES, serum total and free PSA concentrations, prostate volume and PSA density (serum PSA/ prostate volume) were observed (Table: 7,8, and 9). Serum total PSA and free PSA and PSA percent was unaltered by the treatments. Although at 6-months, greater prostate volume was observed in the SPI (-) group as compared to the MPI group, PSA density did not differ between groups (Tables 7 and 8).

Prostatic Bax expression was lower in SPI (-) group as compared to MPI group (P = 0.03) and tended to be lower in the SPI (+) group as compared to the MPI group (P =0.10) after 6-months of interventions. PCNA expression was reduced from baseline in the SPI (-) group, however no group differences were observed at 6-months. No changes in prostatic Bcl-2 and EGFr expression or Bax:PCNA /Bax:Bcl-2 ratio were observed (Table 9).

Table 7: Prostate volume and PSA density differences from baseline

	SPI+ n = 10	**SPI-** n = 13	**MPI** n = 15	**P-Value**
Prostate Volume (cm^3)				
Baseline	52 ± 5	47 ± 5	54 ± 6	0.6709
6 Mos Change	-4.3 ± 3 *ab*	1.6 ± 2 *a*	-5.5 ± 2 *b*	0.0951
PSA Density (ng/mL/cc)				
Baseline	0.1 ± 0.03	0.09 ± 0.02	0.1 ± 0.02	0.8255
6 Mos Change	0.0001 ± 0.01	-0.003 ± 0.01	-0.005 ± 0.01	0.9614

Baseline data are unadjusted means ± standard errors. Differences are post-intervention minus baseline and are least-squares means adjusted for baseline measurement ± standard errors. Pre-planned treatment pairwise comparisons are between groups within each row: means that do not share letters are significantly different ($p < 0.05$).

Table 8: Serum PSA differences from baseline

	SPI+ n = 20	SPI- n = 20	MPI n = 18	
Total PSA (ng/mL)				
Baseline	5.4 ± 1	5.0 ± 1	5.1 ± 1	0.9611
3 Mos Change	-0.8 ± 0.5	-0.8 ± 0.5	-0.6 ± 0.6	0.9373
6 Mos Change	-0.5 ± 0.6	-0.8 ± 0.6	-0.2 ± 0.6	0.7880
Free PSA (ng/mL)				
Baseline	0.9 ± 0.09	0.8 ± 0.1	0.9 ± 0.2	0.7259
3 Mos Change	-0.09 ± 0.09	0.04 ± 0.09	-0.10 ± 0.1	0.4867
6 Mos Change	-0.07 ± 0.07	-0.02 ± 0.07	-0.06 ± 0.07	0.8572
PSA Percent				
Baseline	22 ± 2	19 ± 2	22 ± 2	0.5138
3 Mos Change	-0.21 ± 1	0.67 ± 1	-0.74 ± 1	0.6055
6 Mos Change	1.03 ± 1	1.18 ± 1	-0.22 ± 1	0.7196

Baseline data are unadjusted means ± standard errors. Differences are post-intervention minus baseline and are least-squares means adjusted for baseline measurement ± standard errors. Pre-planned treatment pairwise comparisons are between groups within each row: means that do not share letters are significantly different ($p < 0.05$).

Sample sizes listed at column headings are at baseline. At 3 mos, MPI: $n = 17$; at 6 mos, SPI+: $n = 18$; SPI- $n = 19$, MPI: $n = 18$

Table 9: Antigen expression				
HSCORE	**SPI+** n = 14	**SPI-** n = 14	**MPI** n = 13	
Bax				
Baseline	1.38 ± 0.08	1.45 ± 0.07	1.35 ± 0.06	0.6131
6 Mos	1.41 ± 0.06 *ab*	*1.27 ± 0.05 *a*	1.44 ± 0.06 *b*	0.0818
PCNA				
Baseline	1.61 ± 0.1	1.93 ± 0.1	1.86 ± 0.1	0.1494
6 Mos	1.69 ± 0.1	*1.57 ± 0.1	1.81 ± 0.1	0.4107
Bcl-2				
Baseline	1.11± 0.03	*1.17 ± 0.07	1.09 ± 0.03	0.4629
6 Mos	1.15 ± 0.04	*1.15 ± 0.04	1.19 ± 0.04	0.7195
EGFr				
Baseline	1.34 ± 0.08	1.42 ± 0.10	1.39 ± 0.11	0.8264
6 Mos	1.36 ± 0.06	1.37 ± 0.06	1.33 ± 0.06	0.8342
Bax: Bcl-2 ratio				
Baseline	1.25 ± 0.07	1.30 ± 0.10	1.23 ± 0.06	0.8559
6 Mos	1.20 ± 0.05	1.14 ± 0.05	1.22 ± 0.05	0.4806
Bax: PCNA ratio				
Baseline	0.875 ± 0.05	0.758 ± 0.05	0.760 ± 0.05	0.1826
6 Mos	0.894 ± 0.05	0.823 ± 0.05	0.839 ± 0.05	0.6111

Baseline data are unadjusted means ± standard errors. All other data are least-squares means adjusted for baseline measurement ± standard errors. Pre-planned treatment pairwise comparisons are between groups within each row: means that do not share letters are significantly different ($p < 0.05$).
The number of patients evaluated for bax expression was 16 for SPI-, and 14 for MPI; PCNA expression was 13 for SPI-, and 12 for MPI; Bcl-2 expression was 16 for MPI; EGFr expression was 15 for SPI+; Bax: bcl-2 ratio was 13 for SPI+; Bax: PCNA ratio was 13 for SPI+, 13 for SPI-, and 12 for MPI.

Cancer Incidence: The incidence of prostate cancer (6% in the SPI (+) group, 6% in the SPI (-) group and 38% in the MPI group) was more than 6 times higher in the MPI versus both soy groups ($P = 0.013$).

Effect of equol excretor status on serum hormones and urinary estrogen metabolites: Individuals whose urinary equol concentration exceeded 1000 nmol/day were classified as equol excretors. At 3-months, 4 individuals were classified as equol excretors and 15 as non-excretors in the SPI (+) group. However, at 6-months, only one of these four individuals remained as an equol excretor. Hence comparisons between equol-excretors and non-excretors were only made at the 3-months time-point.

No baseline differences in anthropometrics, dietary intake and cancer status were observed between equol excretors and non-excretors. However, baseline urinary 2:16 OH-E1 concentrations tended to be higher in excretors. After 3-months of SPI (+) intake, serum hormone concentrations and urinary estrogen metabolite levels did not differ between equol excretors and non-excretors.

Effects of soy consumption on isoflavonoid excretion:
Urinary isoflavonoid concentrations from 58 participants were measured by LCMS.

Table 10. Isoflavonoid concentration in 24-hour urine (nmol/day)

	Baseline Median (Range)	**Month-3 Median (Range)**	**Month-6 Median (Range)**
Soy (+)			
	N =20	*N = 19*	*N =17*
ODMA	15 (1, 1372)	11,677 (11, 31985)	11208 (14, 28840)
Equol	54 (9 ,595)	85 (13, 23570)	96 (15, 13500)
Dihydrodaidzein	70 (10, 2379)	12,600 (88, 33803)	15430 (2275, 29365)
Daidzein	295 (51, 25045)	22596 (11604, 45108)	25039 (9311, 46066)
Genistein	125 (13, 21774)	10785 (7662, 26199)	11200 (5001, 42200)
Glycitein	19 (4, 3420)	4301 (1853, 12942)	4979 (1789, 7948)
Soy (-)			
	N =20	*N = 20*	*N =19*
ODMA	16 (6, 2950)	90 (6,1524)	154 (4, 940)
Equol	64 (8, 158)	71 (8, 133)	43 (8, 166)
Dihydrodaidzein	58 (7, 3731)	710 (8, 2474)	482 (4, 2390)
Daidzein	552 (11,7854)	2122 (728, 5215)	2160 (12, 4080)
Genistein	122 (3, 1861)	886 (371,4248)	700 (12, 3024)
Glycitein	20 (3, 858)	152 (9, 799)	165 (8, 340)
Milk			
	N =18	*N = 17*	*N =18*
ODMA	32 (7, 668)	21 (6, 2006)	85 (4, 1551)
Equol	45 (14, 309)	63 (10, 163)	78 (4, 246)
Dihydrodaidzein	27 (7, 380)	21 (6, 1958)	24 (7, 2234)
Daidzein	643 (11, 2866)	380 (15, 5243)	704 (7, 7222)
Genistein	121 (5, 892)	105 (8, 585)	104 (7, 1662)
Glycitein	32 (5, 345)	44 (8, 599)	37 (6, 1329)

The projected completion of the pilot studies in the SoyCaP trial is for August 2007.

Data for the main SoyCaP endpoints i.e. serum hormone concentrations, urinary estrogen metabolites and tissue markers have been analyzed and manuscripts are in press.

Key Research Accomplishments:

Effects of SPI (+):

- No effects on circulating hormone concentrations
- Decreased serum SHBG levels
- Decreased prostatic androgen receptor expression, no effect on estrogen-receptor beta expression
- Increased 24-hour urinary estradiol and concentrations
- Higher 2:16 OH estrone ratio as compared to the MPI group
- No effect on prostate cancer tissue biomarker

Although SPI (+) had no effects on circulating hormone concentrations and decreased SHBG levels (which would theoretically increase androgen availability), prostatic AR expression was lowered. Additionally, increases in urinary estradiol and estrone concentrations and an elevation in the 2:16-OH estrone ratio have been associated with reduced prostate cancer risk. Overall, the effects observed with the SPI (+) interventions are consistent with a protective effect of SPI (+) against prostate cancer.

Effects of SPI (-)

- Increased circulating androgen (androstenedione, DHEAS) and estrogen (estradiol, estrone) concentrations
- Tended to decrease androgen receptor expression, no effects on estrogen-receptor beta expression.
- Decreased serum SHBG levels
- Increased 24-hour urinary estradiol and estrone concentrations
- Reduced prostatic Bax (a protein that is pro-apoptotic) and PCNA (a protein which is a marker of cell proliferation) expression.

Effects of SPI (-) on study endpoints were mixed, with some considered detrimental (reduced Bax expression, decreased serum SHBG concentrations, increases in circulating androgen concentrations) and others beneficial (decreases in PCNA expression, increases in urinary estrone and estradiol levels). It is important to note that although circulating levels of androstenedione and DHEAS increased, serum testosterone levels remained unchanged.Also, prostatic AR expression tended to decrease. Overall, the effects observed with the SPI (-) intervention are consistent with a neutral effect of SPI (-) on prostate cancer prevention.

Effects of MPI:

- No effects on circulating hormone concentration
- Decreased serum SHBG level
- Decreased urinary 2-OH estradiol concentrations
- No effects on prostatic androgen receptor and estrogen-receptor beta expression
- No effect on prostate cancer tissue biomarker

Consistent with its use as a control, most study endpoints were unaltered with the MPI intervention. Decreases in serum SHBG were also observed in the soy groups and were likely due to the increased protein intake observed in all 3 groups.

REPORTABLE OUTCOMES

Grant received:

University of Minnesota Cancer Center Prevention and Etiology Grant "Proteomics for SoyCAP trial" 7/1/05-6/30/07

Papers published:

Hamilton-Reeves J and Kurzer MS. (2003) Effects of soy isoflavone consumption on reproductive hormones in males. *Soy Connection* 11(4): 3-5.

Hamilton-Reeves JM, Rebello SA, Thomas W, Slaton JW, and Kurzer MS. (2007) Soy protein isolate suppresses androgen receptor expression without altering estrogen receptor beta expression or serum hormonal profiles in men at high risk of prostate cancer. *J Nutr*, in press.

Hamilton-Reeves JM, Rebello SA, Thomas W, Kurzer MS and Slaton JW. Effects of soy protein isolate consumption on prostate cancer biomarkers in men at high risk of prostate cancer: results from the SoyCap trial. *Cancer Epidemiol Biomarkers Prev*, submitted.

Hamilton-Reeves JM, Rebello SA, Thomas W, Slaton JW, and Kurzer MS. Soy protein isolate increases urinary estrogens and the ratio of 2:16α-hydroxyestrone in men at high risk of prostate cancer. *Cancer Epidemiol Biomarkers Prev,* submitted.

CONCLUSIONS

Soy protein consumption exerts beneficial effects on estrogen metabolism and steroid hormone receptor expression, potential mediators of prostate cancer preventive effects. In this small 6 month study, significantly fewer of the soy protein consumers progressed to cancer than the milk protein consumers. This suggests that a larger phase III clinical trial of soy protein in men at high risk of prostate cancer is warranted, with cancer as an outcome.

REFERENCES None

APPENDICES None